# PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

## Improvements in or relating to Bacteriological Testing

We, EVANS MEDICAL LIMITED, of Speke, Liverpool 24, England, a British Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to a tablet for use in bacteriological testing.

It is well known to determine the most suitable antibiotic or chemotherapeutic agent to use in the treatment of a disease by growing bacteria obtained from a patient on a bacteriological nutrient plate. Freshly prepared solutions of antibiotics or chemotherapeutic agents are placed in cavities in the surface of the nutrient plate or in open ended cylinders on its surface and the plate is incubated at a suitable temperature in the usual manner. After some time, the plate is removed from the incubator when it will be seen that the bacteria have grown over the surface of the plate, but clear zones of various diameters will be observed round some of the antibiotics or chemotherapeutic agents. The bacteria are unable to grow in these clear zones owing to the influence of the antibiotic or chemotherapeutic agent. The presence or absence of such zones will show whether or not the bacteria are sensitive to the antibiotics tested. The antibiotic(s) producing the largest zone of inhibition of growth is therefore the agent most suitable for treating the disease affecting the patient from whom the bacteria were obtained.

Various means have been devised to simplify this test by avoiding the necessity of preparing solutions of antibiotics or chemotherapeutic agents and placing them in cavities or cylinders. For example, absorbent paper discs have been treated with antibiotic or chemotherapeutic solutions and dried. These discs can be used instead of the solutions of the antibiotics or chemotherapeutic agents in the test described above. Using such a method it is no longer necessary to form cavities in the nutrient plate or to use cylinders since the discs may be placed directly on the surface of the nutrient plate. Alternatively, compressed tablets, consisting of the antibiotic or chemotherapeutic agent and a solid diluent can be used instead of the paper discs. Many substances have been proposed as bases for such tablets, but they suffer from one or more of the following disadvantages.

i) They react with the antibiotic or chemotherapeutic agent and destroy it or curtail its stability.

ii) They absorb the antibiotic or chemotherapeutic agent so strongly that it is not released in the test, or is released in an irregular manner so that no clear zones or only small and irregular zones are produced.

iii) They interfere with the characteristic activity of the antibiotic and prevent or reduce the formation of zones of inhibition on growths of normally susceptible bacteria.

iv) The base, although it may be inert and non-reactive to many bacteria may not necessarily be inert to all bacteria or to mixtures of bacteria. By way of example may be quoted lactose, a commonly used inert diluent for tablets. This does not inhibit the growth of any common bacteria under normal conditions. If, however, a bacteriological plate has a mixture of Ps Pyocyanea and Staph. Pyogenes or Str. Faecalis growing under certain conditions on its surface and tablets of lactose are placed on it and incubated, then clear zones will be produced as described by Pamela M. Waterworth in Journal Med. Lab. Technology p.p. 245—248 Vol. 15 October 1958. This effect can also be demonstrated with sugars other than lactose.

An object of the present invention is to provide a tablet which can be used for bacterial sensitivity testing as hereinbefore described yet is free from the defects mentioned above.

Accordingly the present invention provides

[*Price 3s. 6d.*]

a tablet for bacteriological sensitivity testing which comprises an oxide of one or more of the metals titanium, zirconium, hafnium, thorium and cerium and an antibiotic or chemotherapeutic agent.

Titanium dioxide is particularly suitable since standards of purity exist for it in the British Pharmaceutical Codex 1959 and it is readily available at a fairly low cost. However, titanium dioxide is not satisfactory as a base for the antibiotics Streptomycin, Neomycin, Kanamycin and Oleandomycin.

The tablet may also contain a hydrophyllic agent such as cellulose, to cause moisture from the nutrient jelly on which the bacteria are growing to enter the tablet so enabling the antibiotic or chemotherapeutic agent to diffuse out. Agar may be used to bind the powders together to form granules for tabletting.

In a preferred embodiment of the invention a base for a tablet for bacterial sensitivity testing is prepared by taking 90 parts of metallic oxide, sterilised by heating at 150°C. and 10 parts of pure cellulose sterilised in a similar manner and mixing them thoroughly. Sufficient of a sterile 2% solution of agar in distilled water is added to form a lightly cohesive mass. This mass is forced through a suitable sterile sieve (about 12 mesh) to form granules. The granules so formed are dried to a low moisture content in an oven at 60°C. When sufficiently dry, the granules are again sifted, preferably through a sterile 20 mesh sieve. These base granules are mixed with a suitable dilution of antibiotic or chemotherapeutic agent, the dilution being made by either milling together the antibiotic or chemotherapeutic agent and the metallic oxide or by freese drying a solution of the antibiotic or chemotherapeutic agent containing metallic oxide in suspension.

A small proportion of lubricant, for example 1% or 2° of Magnesium or Aluminium Stearate, is added to enable the tablets to be ejected from a tablet machine. The granules are compressed to produce tablets for bacterial sensitivity testing.

It has been found by experiment that tablets made by this method are:—

i) More uniform than known tablets: the metallic oxides used (in the presence of cellulose) do not prevent the antibiotics or chemotherapeutic agents diffusing evenly and uniformly to produce their characteristic zones.

ii) The base or bases do not inhibit or interfere with the growth of bacteria; this has been shown by the testing of tablets prepared with the base and without antibiotic or chemotherapeutic agent.

iii) Stable: the metallic oxides used do not react with or inactivate any of the antibiotics or chemotherapeutic agents for which they are used as bases.

iv) A number or other identification symbol can be impressed on the tablet and still remain legible after a test has been carried out. (Lactose base tablets crumble during incubation so that identification after a test is difficult).

Many chemotherapeutic agents and antibiotics are readily inactivated by moisture. It is therefore necessary to ensure that all the materials used in tabletting these substances contain as little moisture as practicable.

It is not easy to compress tablets containing very low concentrations of moisture, but with this invention there may be adopted a different method in which, taking advantage of the fact that the metallic oxides used do not inactivate these chemotherapeutic agents and antibiotics even at elevated temperature, tablets of these substances are made containing as low a proportion of moisture as is consistent with easy tabletting. The tablets are then heated in an oven for a sufficiently long time to remove moisture. This heat treatment has the effect of enhancing the stability of the tablets.

WHAT WE CLAIM IS:—

1. A tablet for bacteriological sensitivity testing which comprises an oxide of one or more of the metals titanium, zirconium, hafnium, thorium and cerium and an antibiotic or chemotherapeutic agent.

2. A tablet as claimed in claim 1 containing in addition a hydrophyllic agent.

3. A tablet as claimed in claim 2 wherein the hydrophyllic agent is cellulose.

4. A tablet as claimed in any of claims 1 to 3 wherein the tablet contains agar used as a binding agent in tabletting.

5. A tablet substantially as described.

6. A tablet for bacteriological sensitivity testing comprising an antibiotic or chemotherapeutic agent and a base consisting of 90 parts by weight of an oxide of one or more of the metals titanium, zirconium, hafnium, thorium and cerium and 10 parts by weight of cellulose.

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PROVISIONAL SPECIFICATION

## Improvements in or relating to Bacteriological Testing

We, EVANS MEDICAL LIMITED, of Speke, Liverpool, 24, England, a British Company, do hereby declare the invention to be described in the following statement:—

This invention relates to bacteriological testing.

It is well known to determine the most suitable antibiotic or chemotherapeutic agent to

use in the treatment of a disease by growing bacteria obtained from a patient on a bacteriological nutrient plate. Freshly prepared solutions of antibiotics or chemotherapeutic agents are placed in depressions in the surface of the plate and the plate is incubated at a suitable temperature in the usual manner. After some time, the plate is removed from the incubator when it will be seen that the bacteria have grown over the surface of the plate, but clear zones of various diameters will be observed round some of the antibiotics or chemotherapeutic agents. The bacteria are unable to grow in these clear zones owing to the influence of the antibiotic or chemotherapeutic agent. The largest zone can be determined by inspection and this zone will be situated round the substance which has the greatest inhibitory effect on the bacteria under test; this substance is therefore the agent of choice in treating the disease affecting the patient from whom the bacteria were obtained.

Means have been devised to simplify this test avoiding the necessity of preparing solutions of antibiotics or chemotherapeutic agents and placing them in cavities. For example, absorbent paper discs have been treated with antibiotic and chemotherapeutic solutions and dried. These discs can be used instead of the solutions of antibiotics and chemotherapeutic agents in the cavities in the nutrient plates. Alternatively, compressed tablets consisting of the antibiotic or chemotherapeutic agent with an inert substance can be used instead of the paper discs. Many substances have been proposed as bases for such tablets, but they suffer from one or more of the following disadvantages:

i) They react with the antibiotic or therapeutic agent and destroy it or prevent it from being stable for more than a very short time,

ii) They absorb the antibiotic or chemotherapeutic agent so strongly that it is not released in the test, or is released in an irregular manner so that no clear zones or only small and irregular sized zones are produced,

iii) The base, although inert and non-reactive to many bacteria may not necessarily be inert to all bacteria or to mixtures of bacteria. By way of example may be quoted Lactose, a commonly used inert base for tablets. This does not inhibit the growth of any common bacteria under normal conditions. If however a bacteriological plate has a mixture of Ps. Pyocyanae and Staph. Pyogenes or Str. Faecalis growing under certain conditions on its surface and tablets of Lactose are placed on it and incubated, then clear zones will be produced as described by Pamela M. Waterworth in Journal Med. Lab. Technology, p.p. 245—248 Vol 15 October 1958. This effect can be demonstrated with other sugars.

An object of the present invention is to provide a tablet which can be used for bacterial sensitivity testing as hereinfore described and yet is free from the defects mentioned above.

According to the invention such a tablet comprises an oxide of a metal occurring in Group IV B of the periodic table (after Mendeleeff) and an antibiotic or chemotherapeutic agent. The metals are Titanium, Zirconium, Hafnium, Thalium, or Cerium. Titanium dioxide is preferred since standards of purity exist for it in the British Pharmaceutical Codex and it is readily available at a low price. However, titanium dioxide is somewhat less satisfactory as a base for the antibiotics known as Streptomycin and Neomycin.

The tablet may also contain a hydrophyllic agent such as cellulose, to cause moisture from the nutrient jelly on which the bacteria are growing to enter the tablet so enabling the antibiotic or chemotherapeutic agent to diffuse out. Agar may be used to bind the powders together to form granules.

In a preferred embodiment of the invention, a base for a tablet for bacterial sensitivity testing is prepared by taking 90 parts of Titanium Dioxide, sterilized by heating at 150°C and 10 parts of pure cellulose sterilized in a similar manner and mixing them thoroughly. Sufficient of a sterile 2% solution of agar in distilled water is added to form a lightly cohesive mass. This mass is forced through a suitable sieve to form granules. The granules so formed are dried to a low moisture content in an oven in a current of air at 60°C—100°C. When sufficiently dry the granules are again sifted, preferably through a 30 mesh sieve. These base granules are mixed with a suitable dilution of antibiotic or chemotherapeutic agent, the dilution being made by either milling together the antibiotic or chemotherapeutic agent and the titanium dioxide or by freeze-drying a solution of the antibiotic or chemotherapeutic agent containing titanium dioxide in suspension.

A small proportion of lubricant, for example 1% of Magnesium Stearate, is added to enable the tablets to be ejected from a tablet machine. The granules are compressed to produce tablets for bacterial sensitivity testing.

It has been found by experiment that tablets produced by this method are:

i) more uniform than known tablets; the metallic oxides used (in the presence of cellulose) do not prevent the antibiotics or chemotherapeutic agents diffusing evenly and uniformly to produce their characteristic zones,

ii) more stable,

iii) fully effective: inert tablets containing no antibiotic or chemotherapeutic agent are in fact inert and do not affect any of the bacteria tested,

iv) stable; the metallic oxides used do not react with or inactivate any of the antibiotics or chemotherapeutic agents commonly in use.

v) a number or other identification symbol can be impressed on the tablet and still remain legible after a test has been carried

out; known tablets crumble so that identification after a test is difficult.

Many chemotherapeutic agents and antibiotics are readily inactivated by moisture. It is therefore necessary to ensure that all the materials used in tabletting these substances contain as little moisture as practicable. It is not easy to compress tablets containing very low concentrations of moisture but with this invention there is adopted a different method in which, taking advantage of the fact that the metallic oxides used do not inactivate these chemotherapeutic agents and antibiotics even at elevated temperatures, tablets of these substances are made containing as low a proportion of moisture as is consistent with easy tabletting. The tablets are then heated in an oven for a sufficiently long time to remove moisture. This heat treatment has the effect of enhancing the stability of the tablets.

This treatment is not possible with many of the bases used hitherto since under the above conditions rapid inactivation of the antibiotic or chemotherapeutic agent would occur.

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